

Stomatal studies in the genus *Habenaria* (Orchidaceae)^a

Bhaurav Tukaram Dangat^{1*} & Rajaram Vithoba Gurav¹

Keywords/Mots-clés : anatomy/anatomie, *Habenaria*, Orchids/orchidées, stomata/stomates, Western Ghats.

Abstract

During the present investigation stomatal length, width, aperture size, stomatal density and stomatal index of eighteen species of *Habenaria* were measured. It has been observed that, in the genus *Habenaria*, stomata were distributed on the lower epidermis only that is the leaves are predominantly hypostomatic. In all the species studied the stomata were anomocytic. The maximum length (L) and the maximum width (W) of the stomata were observed on *Habenaria foliosa* var. *foetida* ($L = 77 \pm 0.9 \mu\text{m}$, $W = 63 \pm 2.7 \mu\text{m}$) while the minimum measurements were observed on *H. furcifera* ($L = 33 \pm 1 \mu\text{m}$, $W = 28 \pm 1.3 \mu\text{m}$). Highest stomatal density was observed on *H. furcifera* ($78/\text{mm}^2$) and the lowest density ($20/\text{mm}^2$) in *H. foliosa* var. *foetida*. The stomatal index was highest on *H. furcifera* (37) followed by *H. suaveolens* (35.1) and lowest on *H. foliosa* var. *foetida* (12.7) followed by *H. plantaginea* (15). In respect to stomatal size, stomatal density and stomatal index a negative correlation was observed.

Résumé

Études des stomates chez le genre *Habenaria* (Orchidaceae) – Au cours de l'étude présentée ici la longueur et la largeur des stomates, la taille de leur ouverture, leur densité et l'indice stomatique ont été mesurés chez dix huit espèces d'*Habenaria*. Nous avons observé que, chez ce genre, les stomates sont répartis sur le seul épiderme inférieur, autrement dit que les feuilles sont principalement hypostomatiques. Chez toutes les espèces étudiées les

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stomates sont anomocytiques. La longueur (L) maximum et la largeur (W) maximum des stomates ont été observées chez *Habenaria foliosa* var. *foetida* ($L = 77 \pm 0.9 \mu\text{m}$, $W = 63 \pm 2.7 \mu\text{m}$) tandis que les grandeurs minimales le furent chez *H. furcifera* ($L = 33 \pm 1 \mu\text{m}$, $W = 28 \pm 1.3 \mu\text{m}$). La densité stomatique la plus élevée ($78/\text{mm}^2$) a été observée chez *H. furcifera* et la moins élevée ($20/\text{mm}^2$) chez *H. foliosa* var. *foetida*. L'indice stomatique le plus grand est noté chez *H. furcifera* (37) suivi par *H. suaveolens* (35,1) tandis que l'indice le plus faible l'est chez *H. foliosa* var. *foetida* (12,7) et *H. plantaginea* (15). On observe une corrélation négative entre la taille des stomates, la densité stomatique et l'indice stomatique : les deux derniers décroissent quand la première augmente.

Introduction

Stomata are minute apertures or tiny pores surrounded by a pair of guard cells with or without subsidiary cells, on the lower or upper epidermis of a leaf. Anatomical dermal characters are considered as good indicators of habitat quality, since they manifest variability in relation to microclimatic conditions (Barber *et al.*, 2004). Both stomata and stomatal characters (stomata number, size, stomatal density, stomatal apparatus and guard cell architecture) respond to environmental and physiological cues (Nadeau & Sack, 2002; Gitz & Bakern, 2009). Long time adaptation is indicated by a change in stomata number whereas short time adaptation is characterized by an adjustment of the apertures (Roelfsema & Hedrich, 2005; Casson & Gray, 2008). Whereas stomatal characters, such as size, density frequency and their relationship to changing environmental conditions in general, have been reported frequently, these characteristics have hitherto not been studied in respect to Indian orchids, especially to *Habenaria*. Inamdar (1968), Williams (1979), Singh (1981), Kaushik (1983) and Samuel & Bhat (1994) reported that leaves in the Habenariinae are predominantly hypostomatic. The same authors as well as Das & Paria (1992) reported anomocytic stomatal apparatus whereas Stern (1997) stated that the tribe Orchideae is the only major orchid group in which subsidiary cells do not occur.

The orchid family is well known for its various adaptations in respect to seed germination and seed dispersal. The species of the genus *Habenaria* that grow mainly in the rainy seasons also show great adaptations in

respect to growth pattern and related physiology. In this study, we have examined the stomata in 18 species of *Habenaria* in the Western Ghats.

Material and methods

1) Collection: Plant specimens were collected at various localities of the Western Ghats, India. They were cultivated at the Botanical garden of the Department of Botany, Shivaji University, Kolhapur, Maharashtra, India. Of each species, a plant without tuber was preserved in FAA (90 ml Formalin + 5 ml glacial acetic acid + 5 ml 70% ethyl alcohol). After one month this material was transferred to 70% ethyl alcohol. Leaves of *Habenaria brachyphylla* (Lindley) Aitchison, *H. commelinifolia* (Roxburgh) N.Wallich ex Lindley, *H. crinifera* Lindley, *H. digitata* Lindley, *H. diphyllea* (Nimmo) Dalzell, *H. foliosa* A.Richard var. *foetida* (Blatter and McCann) Bennet, *H. furcifera* Lindley, *H. gibsonii* J.D.Hooker, *H. grandifloriformis* Blatter & McCann, *H. heyneana* Lindley, *H. longicorniculata* J.Graham, *H. longicoru* Lindley, *H. marginata* Colebrooke, *H. ovalifolia* R.Wight, *H. plantaginea* Lindley, *H. rariflora* A.Richard, *H. roxburghii* D.Nicolson and *H. suaveolens* Dalzell were selected for the stomatal study.

2) Isolation of leaf epidermal layers: Mature leaves were used to study the dermal characters according to the methods suggested by Williams (1975). As *Habenaria* is hypostomatic, epidermal peels were taken from abaxial surface of leaves. The peels were mounted in glycerine and the slides were sealed with nail polish.

3) Recording the stomatal characters: Photographs were taken using a Carl Zeiss Axio Imager A2 and a Leica 2000DM compound microscope. The number of stomata, the length and width of the aperture and the size and number of epidermal cells were recorded. Ten readings ($n=10$) were taken per species for correct analysis of stomatal index (the percentage of the number of stomata to the total number of epidermal cells), the stomatal density (number of stomata per mm^2 observed in all species) using the method suggested by Salisbury (1927). The stomata of each species are illustrated with line drawings (Plate I and II) and by photographs (Plate III).

Results and Discussion

On all of the species studied, stomata could only be observed on the lower epidermis of the leaves. Our study thus confirms earlier findings regarding the predominately hypostomatic nature of the genus *Habenaria*. On the

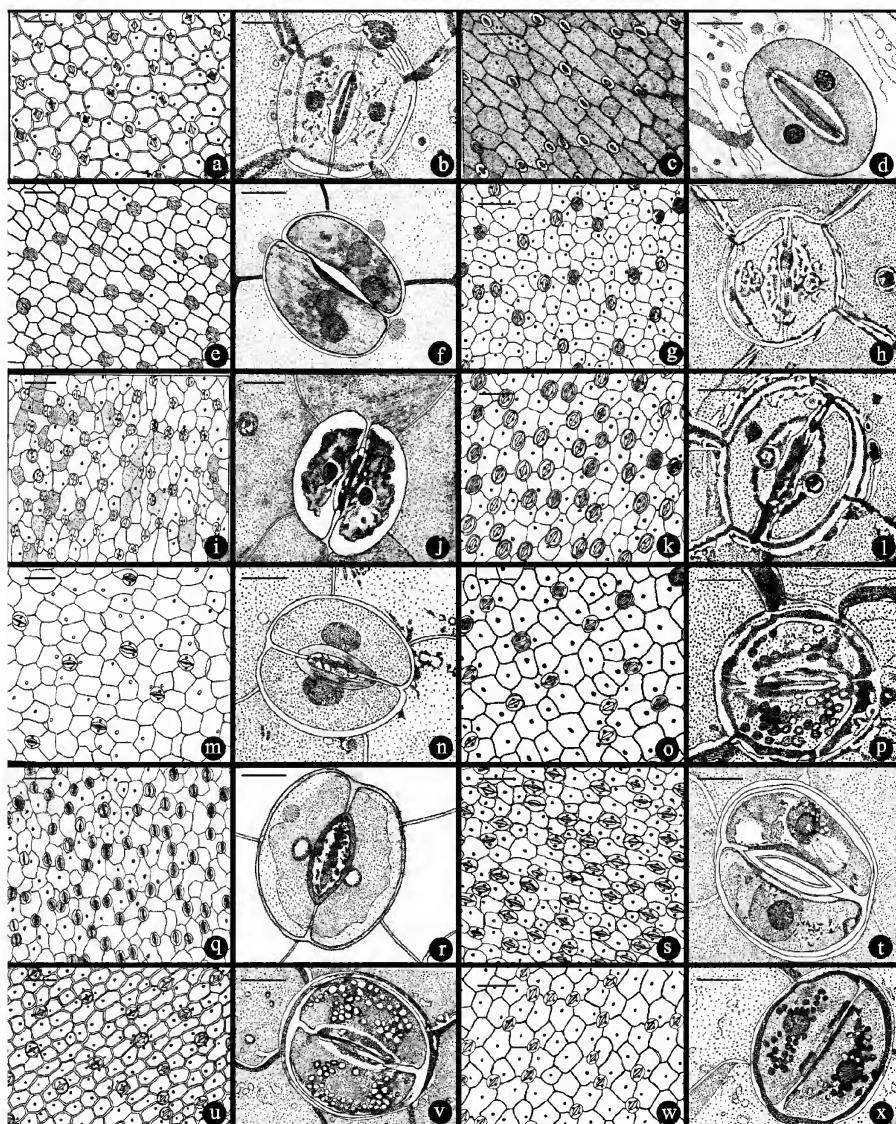


Plate I. Stomata and stomatal apparatus of

Habenaria brachyphylla [a, b]; *H. commelinifolia* [c, d]; *H. crinifera* [e, f]; *H. digitata* [g, h]; *H. diphylla* [i, j]; *H. foliosa* var. *foetida* [k, l]; *H. furcifera* [m, n]; *H. gibsonii* [o, p]; *H. grandifloriformis* [q, r]; *H. heyneana* [s, t]; *H. longicorniculata* [u, v]; *H. longicornu* [w, x]
(Scale bars at 10X: 100 µm, at 100X: 20 µm)

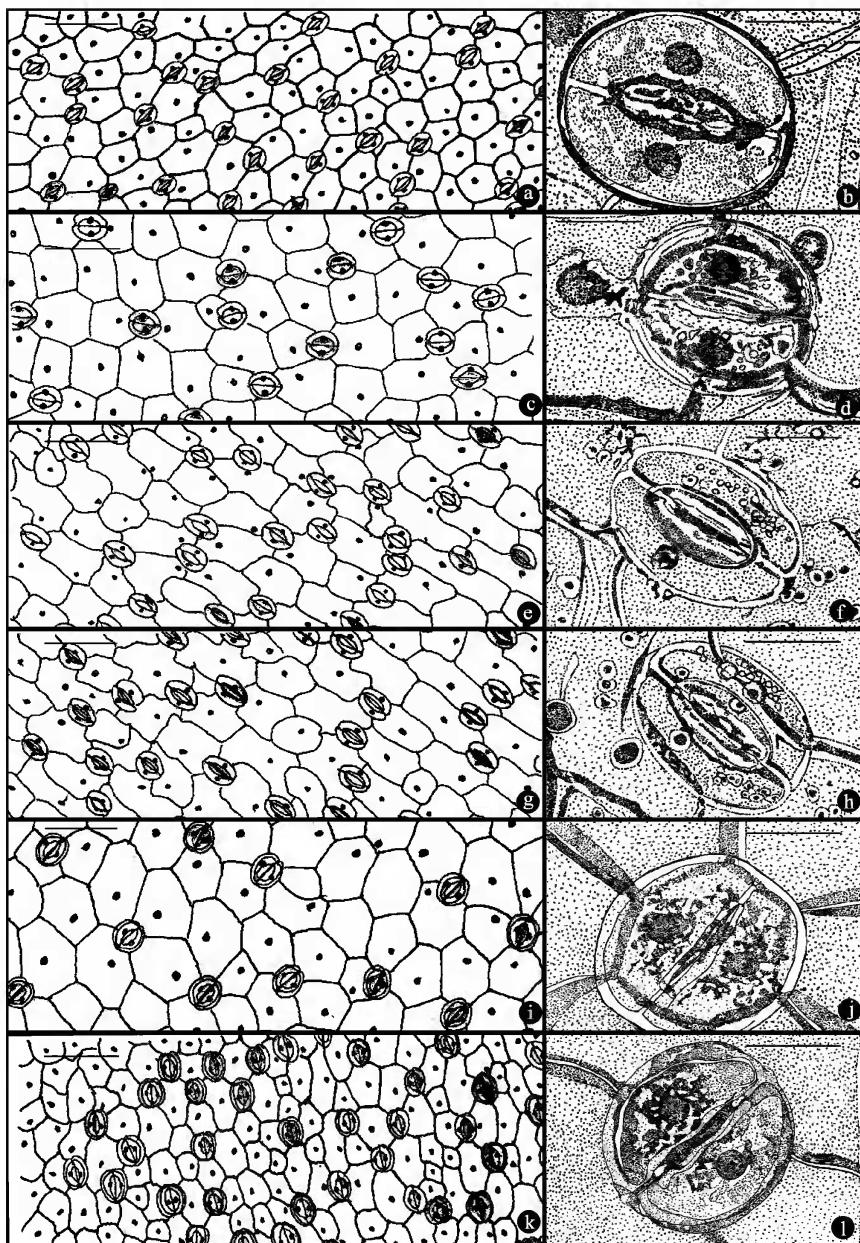


Plate II. Stomata and stomatal apparatus of

Habenaria marginata [a, b]; *H. ovalifolia* [c, d]; *H. plantaginea* [e, f]; *H. rariflora* [g, h]; *H. roxburghii* [i, j]; *H. suaveolens* [k, l] (Scale bars at 10X: 100 µm, at 100X: 30 µm)

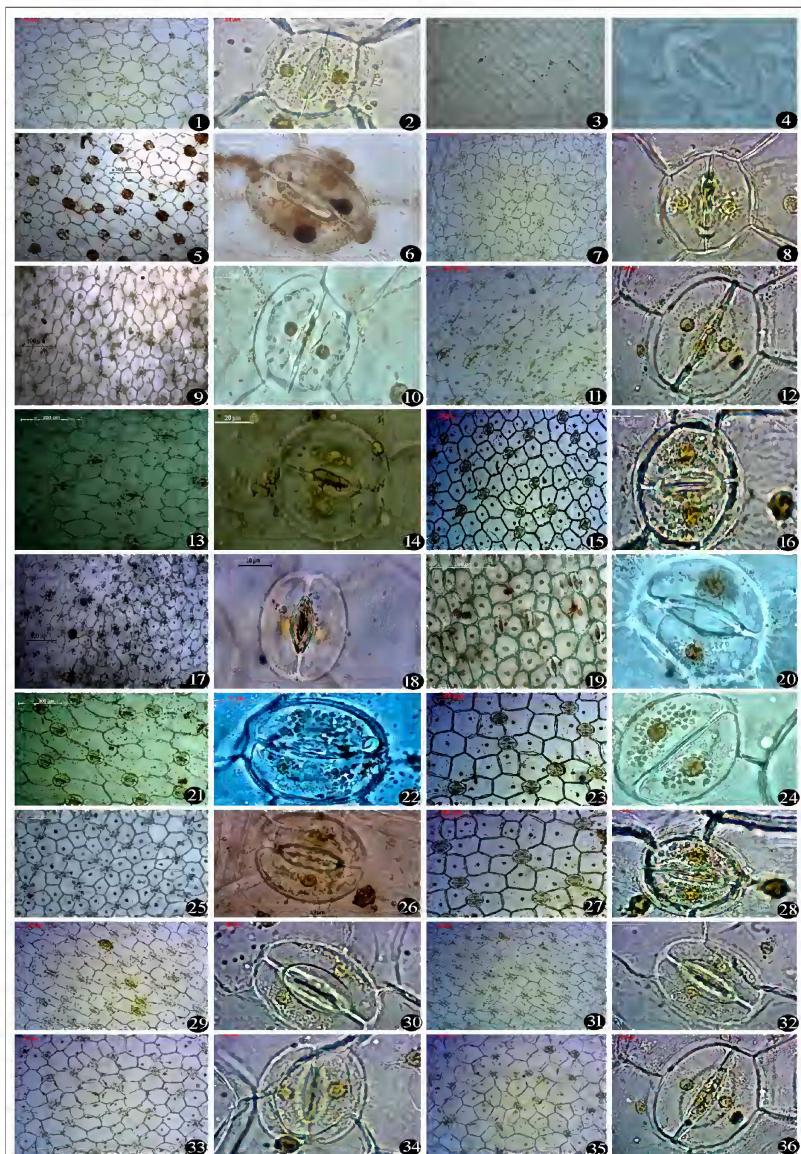


Plate III. Stomatal diversity in the genus *Habenaria*

H. brachyphylla [1, 2]; *H. commelinifolia* [3, 4]; *H. crinifera* [5, 6]; *H. digitata* [7, 8]; *H. diphyllea* [9, 10]; *H. foliosa* var. *foetida* [11, 12]; *H. furcifera* [13, 14]; *H. gibsonii* [15, 16]; *H. grandifloriformis* [17, 18]; *H. heyneana* [19, 20]; *H. longicorniculata* [21, 22]; *H. longicornu* [23, 24]; *H. marginata* [25, 26]; *H. ovalifolia* [27, 28]; *H. plantaginea* [29, 30]; *H. rariflora* [31, 32]; *H. roxburghii* [33, 34]; *H. suaveolens* [35, 36] (at 10X and 100X respectively)

Sr. No.	Species	Stomata		Epidermal Cell	
		L (μm)	W (μm)	L (μm)	W (μm)
1	<i>H. brachyphylla</i>	65 \pm 5	52 \pm 2.2	171 \pm 14	122 \pm 15
2	<i>H. commelinifolia</i>	69.3 \pm 3	54 \pm 2.9	258 \pm 23.3	70 \pm 15.1
3	<i>H. crinifera</i>	61 \pm 1.8	54 \pm 1.3	108 \pm 13	80 \pm 12
4	<i>H. digitata</i>	61 \pm 1.8	54 \pm 1.3	126 \pm 15	67 \pm 11
5	<i>H. diphyllea</i>	49 \pm 1.8	46 \pm 1.3	163 \pm 14	75 \pm 11
6	<i>H. foliosa</i> var. <i>foetida</i>	77 \pm 0.9	63 \pm 2.7	177 \pm 20.8	110 \pm 9.2
7	<i>H. furcifera</i>	33 \pm 1	28 \pm 1.3	118 \pm 12.3	86 \pm 8.2
8	<i>H. gibsonii</i>	52 \pm 2.3	44 \pm 2.1	125 \pm 18	70 \pm 8.5
9	<i>H. grandifloriformis</i>	46 \pm 1	43 \pm 0.7	112 \pm 13	61 \pm 14
10	<i>H. heyneana</i>	48 \pm 2.1	40 \pm 1.3	122 \pm 12.3	69 \pm 9.3
11	<i>H. longicorniculata</i>	60 \pm 20	59 \pm 1	164 \pm 17	69 \pm 9.3
12	<i>H. longicornu</i>	70 \pm 1.5	61 \pm 2	158 \pm 21.3	104 \pm 6.5
13	<i>H. marginata</i>	56 \pm 1.5	45 \pm 0.8	133 \pm 16	74 \pm 6.3
14	<i>H. ovalifolia</i>	57 \pm 2.2	55 \pm 2.2	142 \pm 16.3	76 \pm 10.1
15	<i>H. plantaginea</i>	71 \pm 1	52 \pm 1.1	115 \pm 10.1	74 \pm 6.3
16	<i>H. rariflora</i>	53 \pm 1	44 \pm 0.7	120 \pm 10.1	77 \pm 6.31
17	<i>H. roxburghii</i>	68 \pm 1.1	60 \pm 1	173 \pm 11.4	105 \pm 9.5
18	<i>H. suaveolens</i>	40 \pm 1.2	29 \pm 1	123 \pm 15.1	55 \pm 11

Table 1. Stomatal

other hand we could not confirm the amphistomatic nature of *Habenaria digitata* as reported by Das and Paria (1992). Furthermore we observed that all the species examined during our study had anomocytic stomata without any clear distinction between subsidiary and epidermal cells. (Plate I, II and III). Details regarding the stomatal character variation in the genus *Habenaria* are shown in Table 1. Stomatal length (L) showed a variation from 33 \pm 1 to 77 \pm 0.9 μm , while stomatal width varied from 28 \pm 1.3 to 63 \pm 2.7 μm . The length of epidermal cell varied from 258 \pm 23.3 μm in *H. commelinifolia* (Plate I c and d) to 108 \pm 13 μm in *H. crinifera* (Plate I e and f). Epidermal cell width was highest in *H. brachyphylla* (122 \pm 15 μm ; Plate I a and b) and lowest (55 \pm 11 μm) in *H. suaveolens* (Plate II k and l). We merely observed little variation in respect to the stomatal aperture through which gaseous exchange takes place. The smallest stomatal aperture length (18 \pm 1.3 μm) was observed in *H. furcifera* (Plate I m and n) and the largest (45 \pm 1 μm) in *H. foliosa* var. *foetida* (Plate I k and l). The stomatal aperture was widest in *H. longicornu* (16 \pm 0.9 μm ; Plate I w and x) and narrowest in *H. suaveolens*.

Stomatal aperture		Stomatal density (/mm ²)	Epidermal cell density (/mm ²)	Stomatal Index
L (μm)	W (μm)			
27 ± 1.4	6 ± 1.1	41 ± 4	92 ± 10	30.8
42 ± 1.4	13 ± 2	30 ± 9	135 ± 12	18.2
42 ± 1.40	14 ± 1.3	41 ± 1	134 ± 5	23.4
42 ± 1.40	6 ± 0.9	44 ± 6	138 ± 12	24.2
23 ± 1.8	7 ± 1.3	50 ± 3	127 ± 8	28.2
45 ± 1	12 ± 0.6	20 ± 10	137 ± 11	12.7
18 ± 1.3	8 ± 1.1	78 ± 8	133 ± 21	37.0
39 ± 1.5	11 ± 1.3	48 ± 7	138 ± 16	25.8
25 ± 1.1	12 ± 1	50 ± 4	103 ± 11	32.7
28 ± 1	15 ± 0.9	57 ± 5	189 ± 14	23.2
35 ± 0.9	14 ± 0.9	34 ± 6	194 ± 11	14.9
35 ± 0.9	16 ± 0.9	23 ± 3	94 ± 10	19.7
29 ± 1.6	10 ± 1	47 ± 6	99 ± 20	32.2
35 ± 1.6	10 ± 1	40 ± 9	133 ± 6	23.1
41 ± 1.2	12 ± 1.1	23 ± 8	130 ± 16	15
34 ± 1	11 ± 0.7	48 ± 6	139 ± 13	25.7
33 ± 0.7	11 ± 0.7	25 ± 3	66 ± 11	27.5
19 ± 1.6	5 ± 0.8	65 ± 4	120 ± 9.5	35.1

characters in *Habenaria*

(5 ± 0.8 μm; Plate 2 k and l).

Maximum stomatal density was observed in *H. furcifera* (78 ± 8 per mm²) and lowest stomatal density (20 ± 10 per mm²) was found in *H. foliosa* var. *foetida*. Maximum epidermal cell density was observed in *H. longicorniculata* (194 ± 11 per mm²) and minimum density (66 ± 11 per mm²) in *H. roxburghii*. The stomatal index varied from 12.7 to 37: *Habenaria furcifera* (37) followed by *H. suaveolens* (35.1), *H. plantaginea* (15) and *H. foliosa* var. *foetida* (12.7). A negative correlation was observed between stomatal size, stomatal density and stomatal index. Stomatal density and index decreases as stomatal size increases.

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1 : Department of Botany, Shivaji University, Kolhapur, India
* corresponding author - schndangat@gmail.com